

CLAIMS

1. A method of screening a physiologically active substance, comprising the steps of:

5 (1) contacting a transformed yeast with a test sample, wherein the transformed yeast is capable of expressing a heterogeneous protein, and shows a change in a growing state in an expression state of the protein as compared to that in a non-expression state of the protein;

10 (2) culturing the yeast under conditions that the protein is capable of being expressed; and

(3) measuring the growing state of the yeast,
wherein the physiologically active substance is judged to be present in the test sample in a case where the growth of the yeast is lowered or improved in the presence of the test sample as compared to that in the absence of the test sample
15 as a control.

2. The method of screening according to claim 1, wherein the heterogeneous protein is capable of lowering the growth of the yeast as compared to that of the non-expression state.

20 3. The method of screening according to claim 1 or 2, wherein the heterogeneous protein is a protein involved in regulating cell cycle of a mammal cell.

25 4. The method of screening according to claim 3, wherein the protein

involved in regulating cell cycle of a mammal cell is a protein involved in intracellular signaling of G0/G1 phase of a mammal cell.

5. The method of screening according to any one of claims 1 to 4, wherein
5 the growing state of the yeast is determined in the step (3) by monitoring a change in turbidity of an yeast culture medium, a morphological change of the yeast, a change in wet-weight of the yeast, a change in dry-weight of the yeast, a change in endogenous enzyme activity of the yeast or a change in amount of an endogenous protein of the yeast.

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6. The method of screening according to any one of claims 1 to 5, wherein the transformed yeast is deficient in aspiration ability.

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7. A transformed yeast being capable of expressing a heterogeneous protein, and showing a change in a growing state in an expression state of the protein as compared to that in a non-expression state of the protein.

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8. The transformed yeast according to claim 7, wherein the change in the growing state is lowering of the growth.

9. The transformed yeast according to claim 7 or 8, wherein the heterogeneous protein comprises at least an active site of the protein in an active state.

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10. The transformed yeast according to any one of claims 7 to 9, wherein the

heterogeneous protein is a protein involved in regulating cell cycle of a mammal cell.

11. The transformed yeast according to claim 10, wherein the protein
involved in regulating cell cycle of a mammal cell is a protein involved in
intracellular signaling of G0/G1 phase of a mammal cell.

12. The transformed yeast according to claim 11, wherein the protein
involved in intracellular signaling of G0/G1 phase of a mammal cell is a protein
belonging to Tob family and/or a protein belonging to Caf family.

13. The transformed yeast according to claim 12, wherein the protein
belonging to Tob family is:

(a) a protein comprising in an N-terminal region of the amino acid sequence
thereof the amino acid sequence of SEQ ID NO: 1;

(b) a protein comprising in an N-terminal region of the amino acid sequence
thereof an amino acid sequence having deletion, addition, insertion or
substitution of at least one amino acid residue in the amino acid sequence of
SEQ ID NO: 1, wherein the protein induces growth inhibition of the transformed
yeast in the expression state;

(c) a protein comprising in an N-terminal region of the amino acid sequence
thereof an amino acid sequence having a sequence identity of 20% or more to the
amino acid sequence of SEQ ID NO: 1, wherein the protein induces growth
inhibition of the transformed yeast in the expression state; or

(d) a protein comprising in an N-terminal region of the amino acid sequence

thereof an amino acid sequence having a sequence identity of 20% or more to the amino acid sequence of SEQ ID NO: 2 in a region from an N-terminal to 100 amino acid residues, wherein the protein induces growth inhibition of the transformed yeast in the expression state.

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14. The transformed yeast according to claim 12, wherein the protein belonging to Caf family is:

(a) a protein comprising the amino acid sequence of SEQ ID NO: 4;

10 (b) a protein comprising an amino acid sequence having deletion, addition, insertion or substitution of at least one amino acid residue in the amino acid sequence of SEQ ID NO: 4, wherein the protein induces growth inhibition of the transformed yeast in the expression state; or

(c) a protein comprising an amino acid sequence having a sequence identity of 20% or more to the amino acid sequence of SEQ ID NO: 4, wherein the
15 protein induces growth inhibition of the transformed yeast in the expression state.

15. The transformed yeast according to any one of claims 7 to 14, wherein the transformed yeast comprising a gene encoding a heterogeneous protein operably in expression ligated to a promoter.

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16. The transformed yeast according to any one of claims 7 to 15, wherein the transformed yeast is deficient of aspiration ability, which is used for the method of screening as defined in claim 6.

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17. A physiologically active substance obtainable by the method of screening

as defined in any one of claims 1 to 6.

18. A kit for screening a physiologically active substance, comprising the yeast as defined in any one of claims 7 to 16.